Purpose:Make a gel for analysis and characterization of your purified protein

Make this a day or two before you want to run the gel.

It can be stored at 4OC for 3-5 days.

**Stock Solutions for Polyacrylamide Gels** – these may already be made – check the fridge

1. **40% acrylamide: bisacrylamide (37.5:1**)– stored at 4°C; NEUROTOXIN!!
   * **Be sure not to get the 19:1 solution that Aptamer uses**!
2. **1 M Tris-HCl, pH 8.8:**
   * Measure 60.57 g Tris base (121.14 g/mol)
   * Fill up to ~400 mL with Nanopure H2O and stir until dissolved
   * Add conc. HCl until pH 8.8
   * In graduated cylinder, add Nanopure H2O to give total volume 500 mL
   * Store in 4°C
3. **0.5 M Tris-HCl, pH 6.9:**
   * Measure 15.14 g Tris base (121.14 g/mol)
   * Fill up to ~200 mL with Nanopure H2O and stir until dissolved
   * Add conc. HCl until pH 6.9
   * In graduated cylinder, add Nanopure H2O to give total volume 250 mL
   * Store at 4°C
4. **10% Ammonium Persulfate (Discard after a week):**
   * In a 1.7 mL microcentrifuge tube, add 20 mg ammonium persulfate
   * Add Nanopure H2O to give total volume of 200 uL
   * Store in -20°C
5. **10% SDS**
   * Measure 10g SDS
   * Add Nanopure H2O to give total volume of 100 mL
   * Store at room temperature \*Make sure the SDS bubbles when you shake it!

**Assemble gel hardware and drying stand**

Two glass plates – one notched, one flat

One drying stand with two green plate holders

Well comb (green with teeth) – we use 1mm thick ones

If drying stand is being used, can use tape and binder clips to hold plates together with craft foam on the bottom of plates to hold the liquid in while it dries (polymerizes)

**Important: First:** perform a **Leak Test** on your setup using water. If it leaks, re-do it.

**Make 12% Resolving/Separating Gel: (make before stacking gel)**

|  |  |
| --- | --- |
|  | **Resolving (separating)** |
| **H20** | 2.15 **ml** |
| **1M TrisHCl pH 8.9** | 1.25 **ml** |
| **0.5 M TrisHCl ph 6.8** | 0 |
| **10%SDS** | 50 ul |
| **10% APS** | 31 ul |
| **40% acrylamide:bisacrylamide** | 1.5 **ml** |
| **TEMED** | 10 ul |
|  | **5 ml total volume** |
| This is enough for 1 gel that is 12% | |

**(This is the bottom gel)**

Mix components in a small beaker

\*Make sure you have Pasteur pipet or pipetter ready after adding APS and TEMED because acrylamide can polymerize quickly!

After pipetting enough gel (about ¾ of plate from the bottom), add butanol or 100% ethanol (found in flammable cabinet) to level out top of gel and get rid of bubbles.

When gel is all polymerized – 30 – 45 min, wash out the alcohol from the top with water.

Then proceed to make stacking gel.

|  |  |
| --- | --- |
|  | **Stacking** |
| **H20** | 1.58 **ml** |
| **1M TrisHCl pH 8.9** | 0 |
| **0.5 M TrisHCl ph 6.8** | 625 ul |
| **10%SDS** | 25 ul |
| **10% APS** | 25 ul |
| **40%**  **acrylamide:bisacrylamide** | 250 ul |
| **TEMED** | 5 ul |
| Bromophenol blue powder to help visualize wells | **1 grain** |
|  | **2.5 ml total volume** |

**Make 5% Stacking Gel:**

**(This is the top gel)**

Mix components in a small beaker

\*Pipette quickly on top of previous gel

Insert 10-well comb to gel

Prevent bubbles in gel

Let the gel polymerize (20-30 min)

Once dried:

* Label with your name and date using tape
* Wrap in saran wrap
* Store with combs in place in 4OC fridge (for 3-5 days)

Label with tape and your name and date.

Discard gel solution mix and dried mix in black gel waste tubs

After running gels, wash, rinse and dry plates and place back into original box. **Do not leave them out** – or they will get broken/ lost/stolen….